Oncogenic Transformation by vRel Requires an Amino-Terminal Activation Domain

JOANNE KAMENS,1,2 PAUL RICHARDSON,3 GEORGE MOSIALOS,4 ROGER BRENT,1,2* AND THOMAS GILMORE3

Department of Molecular Biology, Massachusetts General Hospital, 50 Blossom Street, Boston, Massachusetts 02114; Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115; and Departments of Biology3 and Chemistry,4 Boston University, Boston, Massachusetts 02215

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The mechanism by which the products of the v-rel oncogene, the corresponding c-rel proto-oncogene, and the related dorsal gene of Drosophila melanogaster exert their effects is not clear. Here we show that the v-rel, chicken c-rel, and dorsal proteins activated gene expression when fused to LexA sequences and bound to DNA upstream of target genes in Saccharomyces cerevisiae. We have defined two distinct activation regions in the c-rel protein. Region I, located in the amino-terminal half of rel and dorsal proteins, contains no stretches of glutamines, prolines, or acidic amino acids and therefore may be a novel activation domain. Lesions in the v-rel protein that diminished or abolished oncogenic transformation of avian spleen cells correspondingly affected transcription activation by region I. Region II, located in the carboxy terminus of the c-rel protein, is highly acidic. Region II is not present in the v-rel protein or in a transforming mutant derivative of the c-rel protein. Our results show that the oncogenicity of Rel proteins requires activation region I and suggest that the biological function of rel and dorsal proteins depends on transcription activation by this region.

The v-rel oncogene was first identified as the transforming gene of the avian retrovirus reticuloendotheliosis virus T (7, 41, 52). Reticuloendotheliosis virus T causes lymphomas in young chickens (48), and the v-rel protein product (vRel) causes oncogenic transformation of spleen cells in vitro (21). vRel is almost identical to the product of the c-rel proto-oncogene (cRel) over most of its length (Fig. 1) (6a, 19, 52). However, vRel is missing 2 amino-terminal and 118 carboxy-terminal amino acids found in chicken cRel and, instead, contains amino acids derived from the viral env gene (6a, 19, 41, 52).

In addition to transforming spleen cells, vRel can stimulate expression of certain viral promoters transiently transfected into mammalian cells (transactivation [14, 19]). The vRel protein contains a nuclear targeting sequence (16), which is responsible for its localization to the nuclei of chicken embryo fibroblasts (15). However, the relationship between the subcellular location of vRel and its transformation and transactivation functions is not clear. vRel is primarily cytoplasmic in avian spleen cells (15, 38), but mutant vRel proteins that are apparently located exclusively in the cytoplasm or nucleus of spleen cells are also fully transforming (16). vRel and hybrid vRel-cRel proteins in mammalian cells transactivate irrespective of their apparent location in these cells (14, 19).

vRel and cRel share a large region of amino acid similarity with the amino-terminal half of the dorsal protein (Dorsal) (47% conserved [Fig. 1]) (42). The dorsal gene of Drosophila melanogaster was first identified as a maternal-effect embryonic lethal mutation; females homozygous for the dorsal mutation produce embryos that fail to develop ventral structures. The normal function of the wild-type dorsal product in promoting the development of ventral embryonic structures depends on the action of an elaborate cascade of other maternal effect gene products such as Toll, snake, and easter (2, 3). One effect of these gene products is to cause the Dorsal protein to be relocalized from the cytoplasm to the nuclei in the ventral part of the blastoderm (31, 33, 43, 44). Dorsal presumably exerts its effects on development in the nucleus; recent evidence suggests that this may be through regulation of expression of zygotically expressed genes such as zerknult, decapentaplegic, and twist (32, 40, 49).

In light of the evidence that Dorsal may be a regulator of transcription and the observation that Dorsal and Rel proteins are related, we wished to determine whether these proteins function in a similar manner. It has recently become possible to examine the gene regulatory properties of proteins from higher eucaryotes when they are bound to defined DNA sequences located upstream of target genes. Typically, binding to a known DNA site is ensured by expressing the protein as part of a fusion to the DNA-binding domain of the LexA or GAL4 protein (5). These fusion proteins may be expressed in higher eucaryotic cells or in Saccharomyces cerevisiae, in which, in many cases, the ability of activators to interact with the transcription apparatus appears to be conserved (30). Fusion of regulatory proteins to LexA can circumvent a requirement for the regulatory protein to interact with other cellular proteins in order to bind to DNA (5, 26). Experiments with fusion proteins differ from transactivation experiments in that the effects of fusion proteins on transcription can be definitely attributed to their action when bound to sites on DNA upstream of the reporter gene. This approach has been useful in the dissection of activation regions in regulatory proteins (22), in the analysis of oncoprotein function (26, 46, 51; K. Lech et al., unpublished data), and in the examination of proteins involved in Drosophila development (13, 18, 34).

Here we report that DNA-bound Rel and Dorsal proteins activate transcription in S. cerevisiae. We have found that cRel contains two activation domains, region I and region II, whereas vRel contains only region I. cRel does not cause oncogenic transformation of avian spleen cells when it is intact, but does so if region II is deleted. vRel is highly oncogenic, but lesions in region I block this oncogenicity.

* Corresponding author.
Activation region I contains the entire region of high similarity between Rel and Dorsal proteins (Fig. 1). This fact suggests that activation by Rel occurs via a region I, although it is possible that Dorsal contains other activation domains (see Discussion). In summary, these results show that oncogenic transformation by Rel proteins requires activation region I. They further suggest that the function of Dorsal in formation of ventral embryonic Drosophila structures may depend on transcription activation by region I.

**MATERIALS AND METHODS**

Strains, media, and β-Galactosidase Assays. *Escherichia coli* strains used were JM101 [supE thi Δ(lac-proAB) (F' traD36 proAB lacF' ZAM15)], SCS1 [F'' endA1 hsdR17 (trZ trn-1 mK)], and DH5α. All yeast work was done with *S. cerevisiae* BJ2168 (a leu2 trpl ura3-52 pep4-3 prb1-122 prcl-407) (53). Bacteriological work was done by standard techniques (4). Cells were grown in complete minimal 2% glucose media lacking uracil and tryptophan (36). Yeast transformations were done by using the lithium acetate procedure (23). β-Galactosidase assays of liquid cultures were carried out as previously described (18, 20), with at least four individual yeast transformants. β-Galactosidase units are expressed as (1,000 A_420)/(reaction time in minutes) (cell volume in milliliters) (optical density at 600 nm). Assays performed on different days were normalized to values obtained for LexA-vRel.

**Plasmid construction.** DNA manipulations were carried out by standard methods (4). LexA fusion producer plasmids carry the TRP1 gene and CENARS sequences to allow single-copy maintenance in *S. cerevisiae* (11) and were constructed as follows. All rel and dorsal insertions were made at the unique Smal site that cuts after codon 87 of the LexA gene in plasmid pJK1521. pJK1521 is derived from pBC102 (a gift of B. Cormack), which was constructed by insertion of a BamHI fragment from pAAH5 containing a unique HindIII site flanked by the yeast ADH1 promoter and terminator (1) into the BamHI site of pSE358NSM (K. Lech, unpublished data). The HindIII site was converted into an EcoRI site by cutting with HindIII, filling in with the Klenow fragment, and inserting a New England BioLabs linker with the sequence 5'-GGAGAATTCC-3'. The 300-base-pair EcoRI fragment containing the coding sequence for the LexA DNA-binding domain followed by a polylinker was treated with Klenow enzyme to generate blunt ends and was inserted into the EcoRI-cut, Klenow-treated pBC102 vector. The EcoRI sites were destroyed in this manner to facilitate insertion of mutant sequences (see below). In LexA-vRel, all of vRel is present except the initiating methionine. LexA-cRel was fused at a conserved Scal site in the chicken c-rel gene which cuts after amino acid 39; the full-length chicken cRel protein contains 118 Rel amino acids at its carboxy terminus not present in vRel and 2 amino acids at its amino terminus in place of the 11 amino-terminal envelope amino acids in vRel (6a). LexA-Dorsal was made by inserting an SnuBI-AhaIII fragment of dorsal from plasmid dIB6 (a kind gift of R. Steward) which included codons 49 to 677. Codon 49 of dorsal corresponds to codon 18 of v-rel and codon 9 of chicken c-rel. LexA-5' vRel contains amino acids 2 to 331 of vRel and 58 amino acids present after LexA(1-87) in pJK1521. The amino acid sequence after amino acid 87 of the LexA protein is GVRIRIQLWTSGPVEWSSLSQRLS ACMCPQFTKRWKRVKSLLVTLNLNKRISYLD. LexA-3'vRel contains amino acids 331 to 503 of vRel. LexA-cRelXbaI was constructed by inserting a Scal-Klenow-treated XhoI fragment from chicken c-rel at the Scal site of pJK1521. This deletes 55 codons from the 3' end of the c-rel coding sequence. LexA-cRelXbaI therefore contains amino acids 40 to 543 of chicken cRel, followed by amino acids GVPSSNALSDFARGLVSPIVVGGLSTLPFYEKMEKGGQIVGRGTYVDTASK derived from pJK1521 plasmid sequences downstream of the Scal site. LexA-3'cRel is analogous to LexA-3'vRel and contains amino acids 323 to 598 of cRel fused to LexA (1-87). In LexA-VCVRel, a ClaI-HincII fragment was substituted for the corresponding fragment in pLexA-vRel. Chicken cRel
differs by six amino acids from vRel in this substituted region (6a).

To construct the LexA-vRel mutant derivatives, EcoRI fragments from the middle of the v-rel gene containing the indicated alterations were introduced into the LexA-v-rel gene in place of the corresponding wild-type v-rel EcoRI fragment. The construction of the v-rel mutant derivatives (without the fused LexA sequences) in retroviral vectors has been described previously by Gilmore and Temin (16). The approximate positions of the relevant restriction enzyme sites in v-rel are indicated above LexA-vRel in Fig. 4.

Target plasmid pJK101 containing a URA3 gene, a GALI-lacZ fusion gene, and a 2μm replicator was used in all experiments. This plasmid is derived from pLR1Δ20B (50) and contains the high-affinity LexA operator from the promoter of the bacterial ColEl gene (10) inserted into an XhoI site of pBluescribe DNA and extracted from the resulting recombinant plasmid (16).

Retroviral vectors carrying rel genes were constructed as described previously (6a, 16). Construction of the plasmid viral vector, pJD214c-rel, expressing the full-length c-rel gene, is described by Capobianco et al. (6a). The viral vector carrying the c-reldelXbaI gene was constructed by digesting pJD214c-rel with XbaI and religating to remove the small XbaI fragment between the 3' end of the c-rel gene and the polylinker derived from pJD214. JD214c-rel-infected chicken embryo fibroblasts synthesize full-length p68c-rel (6a). Chicken embryo fibroblasts and transformed spleen cells infected with a virus carrying the c-reldelXbaI gene synthesize a correspondingly smaller cRel protein (data not shown). The sequences surrounding serine 275 (ArgArgProSerAsp) and serine 304 (LysArgGluArgSerThr) in vRel suggest that they might be sites for phosphorylation by protein kinase A. Mutations converting the codons for these serines to alanines, 275A and 304A, were made by standard techniques (16, 24) and inserted either into the yeast promoter plasmid as EcoRI fragments as described above or into the avian retroviral vector pJD214 (9), which also carries the wild-type v-rel gene (plasmid pGM282 in Capobianco et al. (6a)). All mutants were confirmed by sequence analysis.

Immunoblot Analysis. To prepare protein extracts, yeast cells in mid-exponential growth phase were centrifuged, suspended in Laemmli sample buffer (25), and immediately frozen at -80°C. Extracts were then heated for 3 min at 100°C and separated on a sodium dodecyl sulfate-9% polyacrylamide gel. Transfer to nitrocellulose, hybridization with anti-LexA antibody, and visualization of LexA protein derivatives were performed as described by Samson et al. (34), except that 3% dry milk was included in the pretreatment buffer and incubation with the anti-LexA antibody was performed for 4 h at room temperature. Equal amounts of protein were loaded in each lane except the LexA-Dorsal lane. It was necessary to load five times more extract from cells synthesizing LexA-Dorsal to visualize the protein.

Spleen cell transformation. Spleen cell transformation data were obtained as described previously (16) or were taken from Gilmore and Temin (16).

RESULTS

vRel, cRel, and Dorsal activate transcription when bound to DNA in yeast cells. To test whether DNA-bound vRel, chicken cRel, and Dorsal activated gene expression, we cotransformed yeast cells with two types of plasmids. Target plasmids carried a GALI-lacZ reporter gene containing an upstream LexA operator. Producer plasmids encoded proteins that contained the DNA-binding domain of the bacterial LexA protein (residues 1 to 87) fused to Rel or Dorsal protein derivatives (see Fig. 3 and 5). Transcription of the GALI-lacZ target gene was measured by assaying β-galactosidase activity. LexA-vRel, LexA-cRel, and LexA-Dorsal proteins of the expected sizes were detected by immunoblot analysis of yeast cell extracts with anti-LexA antiserum (Fig. 2). LexA-Dorsal was expressed at only about 10% of the level of the other proteins.

LexA-vRel, LexA-cRel, and LexA-Dorsal fusion proteins all stimulated expression of the GALI-lacZ target gene (Fig. 3). LexA-vRel directed the synthesis of 190 U of β-galactosidase activity. LexA-cRel stimulated gene expression 10 times more strongly (1,700 U) than LexA-vRel did. LexA-Dorsal stimulated gene expression more weakly (25 U) than LexA-vRel or LexA-cRel; this may be attributable to its low level of expression (Fig. 2). Consistent with this fact is the trend that when LexA-Dorsal is carried on a 2μm-based plasmid and overexpressed in yeast cells, higher levels of target gene expression are observed (J. Kamens, unpublished results). Expression levels were determined relative to the background of 0.1 to 0.5 U of β-galactosidase, observed in cells that expressed only LexA (1–87) (Fig. 3) or nativeLexA (data not shown). Thus, even for LexA-Dorsal there is a 50- to 250-fold stimulation of reporter gene expression. Cells that contained LexA-vRel, LexA-cRel, or LexA-Dorsal did not stimulate the expression of target genes that lacked upstream operators (data not shown).

cRel contains two gene activation domains, one of which is not present in vRel. The region of sequence similarity among vRel, cRel, and Dorsal is restricted to the amino-terminal halves of these proteins (Fig. 1) (19, 42; Capobianco et al., in press). To investigate the functional significance of this region, we constructed hybrid genes that encoded the DNA-binding domain of LexA fused to either the amino terminus...
of vRel (LexA-5'vRel) or the carboxy terminus of vRel (LexA-3'vRel). When synthesized in yeast cells the hybrid proteins were the expected sizes and were present in approximately equal amounts (Fig. 2). As shown in Fig. 3, the amino-terminal region of vRel, which is similar to Dorsal, activated gene expression, whereas the carboxy-terminal region, which contains amino acids specific to Rel proteins, did not. Since the amino-terminal 331 amino acids of vRel are sufficient for gene activation, and since mutations in this region abolished gene activation (see below), we called this region activation region I.

The major difference between cRel and vRel is at their carboxy termini. cRel contains 118 amino acids not present in vRel (19; Capobianco et al., in press). To determine whether this difference was responsible for the high level of gene activation by cRel, we made the following two constructions: we created a truncated cRel protein by deleting 55 amino acids from the carboxy terminus of LexA-cRel (LexA-cReIDelXbal), and we fused the carboxy terminus of cRel directly to LexA (LexA-3'cRel) to create a protein analogous to the LexA-3'vRel construction. LexA-cReIDelXbal activated gene expression much less well than LexA-cRel, at a level similar to that of LexA-vRel (110 U [Fig. 3]). However, the carboxy terminus of cRel activated gene expression very strongly (LexA-3'cRel; 490 U). These results suggest that the carboxy terminus of cRel contains an independent gene activation region (region II, see below) that augments the transcription activation function present in the amino-terminal half of cRel. The idea that it is the carboxy-terminal region that confers heightened transcription activation by cRel is supported by results of an experiment in which we replaced most of activation region I of vRel with the corresponding sequences from cRel (Fig. 3, LexA-VCVRel, 130 U). The level of gene activation was similar to that seen with intact LexA-vRel, indicating that cRel contains an activation region I that is structurally and functionally analogous to that of vRel.

A truncated cRel derivative transforms spleen cells. We define activation region II as the 55 carboxy-terminal amino acids of cRel, since deletion of these amino acids abolished strong transcription activation by cRel. When expressed from a retroviral vector, vRel (which contains activation region I but lacks region II) transforms spleen cells. Full-length cRel, which contains regions I and II, does not do so (Fig. 4). To determine whether the presence of region II is
related to the inability of cRel to transform, we tested truncated cRel (cReldelXbail) in the spleen cell transformation assay. This cRel derivative transformed spleen cells (Fig. 4), demonstrating that region I inhibits transformation by native cRel. cRel that is missing region II thus resembles vRel in both the cell transformation and transcription activation functions. This demonstrates that region II is not necessary for transformation by Rel proteins.

**Transcription activation by region I correlates with cell transformation by vRel.** To examine the relevance of region I to the transforming function of Rel, we tested the effect of mutations in this region on oncogenicity and transcription activation by vRel. Transcription activation was measured with LexA-vRel derivatives. These derivatives lack one amino acid from their amino termini, which has been replaced by the LexA DNA-binding domain. Oncogenicity was measured by a spleen cell transformation assay with vRel derivatives expressed from retroviral vectors; many of these results have been reported previously (16). The LexA moiety was not included on these proteins because it was known that vRel derivatives that carry the LexA moiety or other amino-terminal additions or alterations do not transform in this assay (16, 47; data not shown).

The ability of vRel mutants to transform spleen cells directly correlates with the ability of the corresponding LexA derivatives to activate transcription in yeast cells (Fig. 5). Nontransforming mutants (for example, dStu/Hinc, which is missing amino acids 274 to 331, or TG19, which contains a 15-amino-acid insertion after residue 273) did not activate gene expression. Mutant derivatives which retained transforming activity (for example, NW-1, which contains point mutations in the vRel nuclear localization sequence, or TG18, which contains a 15-amino-acid insertion after amino acid 331) all activated gene expression. A mutant that causes abortive transformation of spleen cells (dHinc/Pvu, which lacks amino acids 332 to 389 and therefore abuts region I [colonies are formed, but do not grow indefinitely]) (16) activated gene expression only weakly. Two mutant derivatives that activated gene expression slightly better than LexA-vRel (dHae/Hinc and TG18NW-1) may have increased transformation activity, but the spleen cell assay is

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<th>Name</th>
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**FIG. 5.** Lesions in activation region I inhibit cell transformation by derivatives of vRel. These altered forms of the LexA-vRel protein were introduced into yeast cells carrying a target plasmid. The approximate positions of the relevant restriction enzyme sites are indicated above LexA-vRel. In-frame deletions are indicated by gaps. The shaded regions represent insertions of a 15-amino-acid sequence corresponding to the simian virus 40 nuclear localization sequence. NW refers to a site-directed mutation that inactivates the major vRel nuclear localizing sequence (16). LexA-275A and LexA-304A have site-directed mutations in serine residues that are consensus sites for phosphorylation by protein kinase A. All hybrid proteins were synthesized in S. cerevisiae and were the expected sizes, as demonstrated by immunoblot analysis with anti-LexA antibody (data not shown). Spleen cell transformation data were obtained for the indicated mutant vRel proteins as expressed from spleen necrosis virus vectors (without the LexA moiety), and data are from Gilmore and Temin (16) or were from assays as described in the legend to Fig. 4 (for 304A and 275A). Symbols: +, transforming; -, nontransforming; +/-, abortive transformation.
not sensitive enough to detect small differences in transforming potential.

The smallest deletion in vRel that inactivated both the transcription activation and cell transformation functions was dStu/Hinc, which lacks 58 amino acids from region I. In all known Rel and Dorsal proteins these amino acids are highly conserved and contain two consensus sites for phosphorylation by protein kinase A (6, 6a, 17, 41, 42, 52). vRel is phosphorylated in vivo (15), and it seemed possible that phosphorylation of these residues (amino acids 275 and 304 in wild-type vRel) would affect transcription activation or oncogenic transformation by vRel (26, 39). However, mutations that changed serines in these protein kinase A consensus recognition sites to alanines (LexA-275A and LexA-304A) did not affect either gene activation or transformation (Fig. 5).

**DISCUSSION**

We have shown that the LexA fusion derivatives of vRel, cRel, and Dorsal proteins stimulate transcription of target genes in yeast cells. Transcription activation depends on the presence of the LexA operator upstream of the target genes, and thus on binding of the fusion protein to the operator (5, 26). cRel is the strongest activator, presumably because it contains an activation domain at its carboxy terminus that is not present in vRel and Dorsal (Fig. 1). This region, region II, is not necessary for transformation by Rel proteins, since it is missing in both vRel and a transforming mutant of cRel. The fact that cRel is such a potent activator of gene expression in yeast cells suggests that activation of transcription is a genuine function of the cRel protein in higher eucaryotes. vRel and Dorsal are weaker activators. All lesions that eliminate transcription activation by region I inactivate the transforming function of vRel. One mutation diminishes transcription activation and diminishes the transforming function of vRel. These results clearly suggest that the region I transcription activation function is required for transformation by Rel proteins.

How do regions I and II activate transcription? In many eucaryotic transcription regulatory proteins, activation regions are characterized by stretches of acidic amino acids, glutamines, or prolines (28, 30, 45). Region II of chicken cRel has a net charge of -7, suggesting that transcription stimulation by it may depend on acidic amino acids. Consistent with this idea, the carboxy termini of mouse and human cRel proteins show no obvious sequence similarity to that of avian cRel, but are all strongly negatively charged (6, 17). Dorsal does not contain a region II, but it does contain stretches of glutamines in its carboxy terminus (42). Glutamine-rich activation domains apparently do not function in S. cerevisiae (C. Besmond, D. Collazo, and R. Brent, unpublished data; G. Gill and R. Tjian, personal communication), but it is possible that these stretches constitute an activation region equivalent to region II when Dorsal is expressed in Drosophila cells. In contrast, region I contains no stretches of acidic amino acids, glutamines, or prolines.

At least three other oncoproteins (Fos, Jun, and Myb) and four other Drosophila developmental proteins (Fitz, Ubx, abd-a, and Bicoid) stimulate gene expression when brought to DNA via fusion to a heterologous DNA-binding domain (13, 18, 26, 34, 46, 51). In each case, it has also been shown that these proteins associate with specific DNA sequences. These results have led to the idea that gene activation by these DNA-bound proteins may be necessary for their roles in oncogenesis and development. Although we have shown that DNA-bound Rel and Dorsal fusion proteins all activate transcription, no specific DNA-binding sites have been identified for the native proteins. Rel proteins are known to associate with a number of cellular proteins (29, 37); if their normal function requires association with specific sites on promoter DNA, it is possible that Rel proteins are bound to DNA via an association with one of these proteins. (A similar model has been proposed for the adenovirus E1A protein, which does not bind DNA directly [12] but activates gene expression when brought to DNA by a heterologous DNA-binding domain [27].)

Recently, Hannink and Temin studied transactivation by vRel derivatives in rat cells (19). They found that all transforming Rel protein derivatives were also able to transactivate expression of the polyomavirus late promoter. However, they found that not all derivatives that transactivate were able to fully transform; they therefore concluded that there is no apparent correlation between the ability of Rel proteins to transactivate the polyomavirus late promoter and the ability of these derivatives to transform. Two facts may account for this apparent discrepancy with our results. First, our assays are different. The effects of Rel proteins on gene expression in our experiments are observed only when the proteins are bound to promoter DNA via LexA moieties, whereas in transactivation assays, the effects of Rel proteins on gene expression are not necessarily due to Rel binding to the promoter (14, 19). Second, in our experiments, transcription activation via region I correlates with oncogenic transformation in vRel derivatives which all lacked region II. Some of the vRel-cRel hybrids used by Hannink and Temin (19) included region II, under which conditions any putative correlation between transactivation and oncogenic transformation might not be observed.

We have shown that region II contains a strong transcription activation function and inhibits transformation by native cRel. It is possible that this region inhibits transformation by intact cRel in some way that is related to its ability to activate transcription, for example by overstimulating expression of some cellular target gene or by titrating some accessory transcription factor. Alternatively, region II may suppress transformation in a manner that is unrelated to its role in transcription activation. For example, recent evidence suggests that the cRel carboxy terminus prevents cRel from entering the nucleus of higher eucaryotic cells (6a, 19). Region II does not always suppress transformation by Rel derivatives. Certain vRel derivatives containing the intact cRel carboxy terminus do transform spleen cells (19; T. D. Gilmore and A. J. Capobianco, unpublished data), indicating that there is more than one mechanism by which the transforming potential of cRel proteins may be activated.

We have shown that the integrity of activation region I is necessary for oncogenic transformation by vRel. This fact is consistent with the idea that the oncogenicity of Rel proteins requires an interaction between region I and some component of the transcription apparatus. We imagine two mechanisms by which such an interaction might be oncogenic. First, transformation by vRel and mutant derivatives might be due to direct stimulation of target genes. This model requires that transforming Rel proteins interact with DNA upstream of target genes, and thus requires that at least a subpopulation of these proteins be present in the nucleus. Alternatively, it is possible that transforming Rel proteins compete with cRel for a component of the transcription apparatus. In this case, transformation would not require an intact region I to activate transcription per se, but, rather, to compete for a transcription factor that normally interacts
with cRel to suppress the malignant state. This hypothesis would predict that transforming Rel proteins would exert a dominant interfering effect on cRel function somewhat akin to the dominant negative effect caused by oncogenic derivatives of erb-B (18, 35). It is worth noting that such a mechanism for Rel transformation would not require that the Rel derivatives be present in the nucleus and would thus explain the fact that apparently cytoplasmic Rel derivatives transform (16). Apparent titration of transcription factors has been observed for GAL4 derivatives, even ones that are not localized to the nucleus (14a, 36a). These GAL4 derivatives presumably sequester a component that normally partitions between the nucleus and the cytoplasm, or sequester a component while it is en route to the nucleus. Finally, it remains possible that some other function of region I, unrelated to its role in transcription activation, is required for Rel to transform spleen cells. However, the strength of the correlation between oncogenic transformation and transcription activation by region I provides a simple hypothesis to guide future work.

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